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Application Number Information

Application Number: **09/335409** [Order This File Assignments](#)

Examiner Number: **73397 / NASHED, NASHAAT**

Filing Date: **06/17/1999**

Group Art Unit: **1652**

Effective Date: **06/17/1999**

Class/Subclass: **435/183.000**

Application Received: **06/17/1999**

Lost Case: **NO**

Patent Number: **6121029**

Interference Number:

Issue Date: **09/19/2000**

Unmatched Petition: **NO**

Date of Abandonment: **00/00/0000**

L&R Code: Secrecy Code: **1**

Attorney Docket Number: **4-30582A**

Third Level Review: **NO**

Secrecy Order: **NO**

Status: **150 /PATENTED CASE**

Status Date: **09/01/2000**

Confirmation Number: **6445**

Oral Hearing: **NO**

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09335409</u>	<u>9200</u>	08/16/2001	No Charge to Location	No Charge to Name	LOE CONV DR1995U	

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L3: Entry 28 of 38

File: USPT

Sep 19, 2000

US-PAT-NO: 6121029

DOCUMENT-IDENTIFIER: US 6121029 A

TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: September 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schupp; Thomas	Mohlin			CH
Ligon; James Madison	Apex	NC		
Molnar; Istvan	Durham	NC		
Zirkle; Ross	Raleigh	NC		
Cyr; Devon Dawn	Fuquay-Varina	NC		
Gorlach; Jorn	Durham	NC		

US-CL-CURRENT: 435/183, 435/189, 435/193, 435/232, 435/252.3, 435/252.35, 435/320.1,
530/300, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes at least one polypeptide required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 21746-43519 of SEQ ID NO:1, nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 30539-30759 of SEQ ID NO:1, nucleotides 30815-32092 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID

NO:1, nucleotides 33401-33889 of SEQ ID NO:1, nucleotides 35042-35902 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, nucleotides 37052-38320 of SEQ ID NO:1, nucleotides 38636-39598 of SEQ ID NO:1, nucleotides 39635-40141 of SEQ ID NO:1, nucleotides 41369-42256 of SEQ ID NO:1, nucleotides 42314-43048 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.

3. A recombinant vector comprising a chimeric gene according to claim 2.

4. A recombinant host cell comprising a chimeric gene according to claim 2.

5. The recombinant host cell of claim 4, which is a bacteria.

6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 30815-32092 of SEQ ID NO:1, and nucleotides 37052-38320 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
9. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.
10. A recombinant vector comprising a chimeric gene according to claim 9.
11. A recombinant host cell comprising a chimeric gene according to claim 9.
12. The recombinant host cell of claim 11, which is a bacteria.
13. The recombinant host cell of claim 12, which is an Actinomycete.
14. The recombinant host cell of claim 13, which is Streptomyces.
15. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID NO:1, and nucleotides 38636-39598 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
16. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 15.
17. A recombinant vector comprising a chimeric gene according to claim 16.
18. A recombinant host cell comprising a chimeric gene according to claim 17.
19. The recombinant host cell of claim 18, which is a bacteria.
20. The recombinant host cell of claim 19, which is an Actinomycete.
21. The recombinant host cell of claim 20, which is Streptomyces.
22. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an enoyl reductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of; nucleotides 35042-35902 of SEQ ID NO:1 and nucleotides 41369-42256 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
23. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 22.
24. A recombinant vector comprising a chimeric gene according to claim 23.

25. A recombinant host cell comprising a chimeric gene according to claim 23.
26. The recombinant host cell of claim 25, which is a bacteria.
27. The recombinant host cell of claim 26, which is an Actinomycete.
28. The recombinant host cell of claim 27, which is Streptomyces.
29. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 30539-30759 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
30. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 29.
31. A recombinant vector comprising a chimeric gene according to claim 30.
32. A recombinant host cell comprising a chimeric gene according to claim 30.
33. The recombinant host cell of claim 32, which is a bacteria.
34. The recombinant host cell of claim 33, which is an Actinomycete.
35. The recombinant host cell of claim 34, which is Streptomyces.
36. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 33401-33889 of SEQ ID NO:1 and nucleotides 39635-40141 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
37. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 36.
38. A recombinant vector comprising a chimeric gene according to claim 37.
39. A recombinant host cell comprising a chimeric gene according to claim 37.
40. The recombinant host cell of claim 39, which is a bacteria.
41. The recombinant host cell of claim 40, which is an Actinomycete.
42. The recombinant host cell of claim 41, which is Streptomyces.
43. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, and nucleotides 42314-43048 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
44. A chimeric gene comprising a heterologous promoter sequence operatively

linked to a nucleic acid fragment according to claim 43.

45. A recombinant vector comprising a chimeric gene according to claim 44.

46. A recombinant host cell comprising a chimeric gene according to claim 44.

47. The recombinant host cell of claim 46, which is a bacteria.

48. The recombinant host cell of claim 47, which is an Actinomycete.

49. The recombinant host cell of claim 48, which is Streptomyces.

50. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:5, amino acids 39-457 of SEQ ID NO:5, amino acids 563-884 of SEQ ID NO:5, amino acids 1147-1399 of SEQ ID NO:5, amino acids 1434-1506 of SEQ ID NO:5, amino acids 1524-1950 of SEQ ID NO:5, amino acids 2056-2377 of SEQ ID NO:5, amino acids 2645-2895 of SEQ ID NO:5, amino acids 2932-3005 of SEQ ID NO:5, amino acids 3024-3449 of SEQ ID NO:5, amino acids 3555-3876 of SEQ ID NO:5, amino acids 3886-4048 of SEQ ID NO:5, amino acid 4433-4719 of SEQ ID NO:5, amino acids 4729-4974 of SEQ ID NO:5, amino acids 5010-5082 of SEQ ID NO:5, amino acids 5103-5525 of SEQ ID NO:5, amino acids 5631-5951 of SEQ ID NO:5, amino acids 5964-6132 of SEQ ID NO:5, amino acids 6542-6837 of SEQ ID NO:5, amino acids 6857-7101 of SEQ ID NO:5, and amino acids 7140-7211 of SEQ ID NO:5.

51. A nucleic acid fragment according to claim 50, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 21746-43519 of SEQ ID NO:1, nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 30539-30159 of SEQ ID NO:1, nucleotides 30815-32092 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID NO:1, nucleotides 33401-33889 of SEQ ID NO:1, nucleotides 35042-35902 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, nucleotides 37052-38320 of SEQ ID NO:1, nucleotides 38636-39598 of SEQ ID NO:1, nucleotides 39635-40141 of SEQ ID NO:1, nucleotides 41369-42256 of SEQ ID NO:1, nucleotides 42314-43048 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1.

52. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising an amino acid sequence selected from the group consisting of: amino acids 39-457 of SEQ ID NO:5, amino acids 1524-1950 of SEQ ID NO:5, amino acids 3024-3449 of SEQ ID NO:5, and amino acids 5103-5525 of SEQ ID NO:5.

53. An isolated nucleic acid fragment according to claim 52, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 30815-32092 of SEQ ID NO:1, and nucleotides 37052-38320 of SEQ ID NO:1.

54. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyltransferase domain comprising an amino acid sequence selected from the group consisting of: amino acids 563-884 of SEQ ID NO:5, amino acids 2056-2377 of SEQ ID NO:5, amino acids 3555-3876 of SEQ ID NO:5, and amino acids 5631-5911 of SEQ ID NO:5.

55. An isolated nucleic acid fragment according to claim 54, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID NO:1, and nucleotides 38636-39598 of SEQ ID NO:1.

56. An isolated nucleic acid fragment according to claim 50, wherein said

polypeptide comprises an enoyl reductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 4433-4719 of SEQ ID NO:5 and amino acids 6542-6837 of SEQ ID NO:5.

57. An isolated nucleic acid fragment according to claim 56, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 35042-35902 of SEQ ID NO:1 and nucleotides 41369-42256 of SEQ ID NO:1.

58. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyl carrier protein domain comprising an amino acid sequence selected from the group consisting of: amino acids 1434-1506 of SEQ ID NO:5, amino acids 2932-3005 of SEQ ID NO:5, amino acids 5010-5082 of SEQ ID NO:5, and amino acids 7140-7211 of SEQ ID NO:5.

59. An isolated nucleic acid fragment according to claim 58, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 30539-30759 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1.

60. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a dehydratase domain comprising an amino acid sequence selected from the group consisting of: amino acids 3886-4048 of SEQ ID NO:5 and amino acids 5964-6132 of SEQ ID NO:5.

61. An isolated nucleic acid fragment according to claim 60, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 33401-33889 of SEQ ID NO:1 and nucleotides 39635-40141 of SEQ ID NO:1.

62. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 1147-1399 of SEQ ID NO:5, amino acids 2645-2895 of SEQ ID NO:5, amino acids 4729-4974 of SEQ ID NO:5, and amino acids 6857-7101 of SEQ ID NO:5.

63. An isolated nucleic acid fragment according to claim 62, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, and nucleotides 42314-43048 of SEQ

ID NO:1.

64. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 50.

65. A recombinant vector comprising a chimeric gene according to claim 64.

66. A recombinant host cell comprising a chimeric gene according to claim 64.

67. The recombinant host cell of claim 66, which is a bacteria.

68. The recombinant host cell of claim 67, which is an Actinomycete.

69. The recombinant host cell of claim 68, which is Streptomyces.

70. An isolated polypeptide required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to a sequence selected from the group consisting of: nucleotides 21746-43519 of SEQ ID NO:1, nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 30539-30759 of SEQ ID NO:1, nucleotides

30815-32092 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID NO:1, nucleotides 33401-33889 of SEQ ID NO:1, nucleotides 35042-35902 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, nucleotides 37052-38320 of SEQ ID NO:1, nucleotides 38636-39598 of SEQ ID NO:1, nucleotides 39635-40141 of SEQ ID NO:1, nucleotides 41369-42256 of SEQ ID NO:1, nucleotides 42314-43048 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

71. A recombinant host cell comprising the polypeptide according to claim 70.

72. The recombinant host cell of claim 71, which is a bacteria.

73. The recombinant host cell of claim 72, which is an Actinomycete.

74. The recombinant host cell of claim 73, which is Streptomyces.

75. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 21860-23116 of SEQ ID NO:1, nucleotides 26318-27595 of SEQ ID NO:1, nucleotides 30815-32092 of SEQ ID NO:1, and nucleotides 37052-38320 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

76. A recombinant host cell comprising the polypeptide according to claim 75.

77. The recombinant host cell of claim 76, which is a bacteria.

78. The recombinant host cell of claim 77, which is an Actinomycete.

79. The recombinant host cell of claim 78, which is Streptomyces.

80. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 23431-24397 of SEQ ID NO:1, nucleotides 27911-28876 of SEQ ID NO:1, nucleotides 32408-33373 of SEQ ID NO:1, and nucleotides 38636-39598 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

81. A recombinant host cell comprising the polypeptide according to claim 80.

82. The recombinant host cell of claim 81, which is a bacteria.

83. The recombinant host cell of claim 82, which is an Actinomycete.

84. The recombinant host cell of claim 83, which is Streptomyces.

85. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an enoyl reductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 35042-35902 of SEQ ID NO:1 and nucleotides 41369-42256 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

86. A recombinant host cell comprising the polypeptide according to claim 85.

87. The recombinant host cell of claim 86, which is a bacteria.
88. The recombinant host cell of claim 87, which is an Actinomycete.
89. The recombinant host cell of claim 88, which is Streptomyces.
90. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 26045-26263 of SEQ ID NO:1, nucleotides 30539-30759 of SEQ ID NO:1, nucleotides 36773-36991 of SEQ ID NO:1, and nucleotides 43163-43378 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
91. A recombinant host cell comprising the polypeptide according to claim 90.
92. The recombinant host cell of claim 91, which is a bacteria.
93. The recombinant host cell of claim 92, which is an Actinomycete.
94. The recombinant host cell of claim 93, which is Streptomyces.
95. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 33401-33889 of SEQ ID NO:1 and nucleotides 39635-40141 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
96. A recombinant host cell comprising the polypeptide according to claim 95.
97. The recombinant host cell of claim 96, which is a bacteria.
98. The recombinant host cell of claim 97, which is an Actinomycete.
99. The recombinant host cell of claim 98, which is Streptomyces.
100. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 25184-25942 of SEQ ID NO:1, nucleotides 29678-30429 of SEQ ID NO:1, nucleotides 35930-36667 of SEQ ID NO:1, and nucleotides 42314-43048 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
101. A recombinant host cell comprising the polypeptide according to claim 100.
102. The recombinant host cell of claim 101, which is a bacteria.
103. The recombinant host cell of claim 102, which is an Actinomycete.
104. The recombinant host cell of claim 103, which is Streptomyces.
105. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:5, amino acids 39-457 of SEQ ID NO:5, amino acids 563-894 of SEQ ID NO:5, amino acids 1147-1399 of SEQ ID NO:5, amino acids 14314-1506 of SEQ ID NO:5, amino acids 1524-1950 of SEQ ID NO:5, amino acids 2056-2377 or SEQ ID NO:5, amino acids 2645-2895 of SEQ ID NO:5, amino acids 2932-3005 of SEQ ID NO:5, amino acids 3024-3449 of SEQ ID NO:5, amino acids

3555-3876 of SEQ ID NO:5, amino acids 3886-4048 of SEQ ID NO:5, amino acids 4433-4719 of SEQ ID NO:5, amino acids 4729-4974 of SEQ ID NO:5, amino acids 5010-5082 of SEQ ID NO:5, amino acids 5103-5525 of SEQ ID NO:5, amino acids 5631-5951 of SEQ ID NO:5, amino acids 5964-6132 of SEQ ID NO:5, amino acids 6542-6837 of SEQ ID NO:5, amino acids 6857-7101 of SEQ ID NO:5, and amino acids 7140-7211 of SEQ ID NO:5.

106. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising an amino acid sequence selected from the group consisting of: amino acids 39-457 of SEQ ID NO:5, amino acids 1524-1950 of SEQ ID NO:5, amino acids 3024-3449 of SEQ ID NO:5, and amino acids 5103-5525 of SEQ ID NO:5.

107. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyltransferase domain comprising all amino acid sequence selected from the group consisting of: amino acids 563-884 of SEQ ID NO:5, amino acids 2056-2377 of SEQ ID NO:5, amino acids 3555-3876 of SEQ ID NO:5, and amino acids 5631-5951 of SEQ ID NO:5.

108. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an enoyl reductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 4433-4719 of SEQ ID NO:5 and amino acids 6542-6837 of SEQ ID NO:5.

109. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyl carrier protein domain comprising an amino acid sequence selected from the group consisting of: amino acids 1434-1506 of SEQ ID NO:5, amino acids 2932-3005 of SEQ ID NO:5, amino acids 5010-5082 of SEQ ID NO:5, and amino acids 7140-7211 of SEQ ID NO:5.

110. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a dehydratase domain comprising an amino acid sequence selected from the group consisting of: amino acids 3886-4048 of SEQ ID NO:5 and amino acids 5964-6132 of SEQ ID NO:5.

111. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 1147-1399 of SEQ ID NO:5, amino acids 2645-2895 of SEQ ID NO:5, amino acids 4729-4974 of SEQ ID NO:5, and amino acids 6857-7101 of SEQ ID NO:5.

112. A recombinant host cell comprising the polypeptide according to claim 105.

113. The recombinant host cell of claim 112, which is a bacteria.

114. The recombinant host cell of claim 113, which is an Actinomycete.

115. The recombinant host cell of claim 114, which is Streptomyces.

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Application Number Information

Application Number: **09/568102** [Order This File Assignments](#)

Examiner Number: **73397 / NASHED, NASHAAT**

Filing Date: **05/10/2000**

Group Art Unit: **1652**

Effective Date: **05/10/2000**

Class/Subclass: **435/183.000**

Application Received: **05/11/2000**

Lost Case: **NO**

Patent Number: **6346404**

Interference Number:

Issue Date: **02/12/2002**

Unmatched Petition: **NO**

Date of Abandonment: **00/00/0000**

L&R Code: Secrecy Code: **1**

Attorney Docket Number: **4-30582B**

Third Level Review: **NO**

Secrecy Order: **NO**

Status: **150 /PATENTED CASE**

Status Date: **01/25/2002**

Confirmation Number: **8431**

Oral Hearing: **NO**

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09568102</u>	<u>9200</u>	07/18/2002	No Charge to Location	No Charge to Name	<u>BAIG,ARIF</u>	

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L3: Entry 19 of 38

File: USPT

Feb 12, 2002

US-PAT-NO: 6346404

DOCUMENT-IDENTIFIER: US 6346404 B1

**** See image for Certificate of Correction ****TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: February 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schupp; Thomas	Mohlin			CH
Ligon; James Madison	Apex	NC		
Molnar; Istvan	Durham	NC		
Zirkle; Ross	Raleigh	NC		
Cyr; Devon Dawn	Fuquay-Varina	NC		
Gorlach; Jorn	Durham	NC		

US-CL-CURRENT: 435/183, 435/189, 435/193, 435/232, 435/252.3, 435/252.35, 435/320.1, 530/350, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes at least one polypeptide required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 7610-11875 of SEQ ID NO:1, nucleotides 7643-8920 of SEQ ID NO:1, nucleotides 9236-10201 of SEQ ID NO:1, nucleotides 10529-11428 of SEQ ID NO:1, and nucleotides 11549-11764 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 7643-8920 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and

washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

9. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.

10. A recombinant vector comprising a chimeric gene according to claim 9.

11. A recombinant host cell comprising a chimeric gene according to claim 9.

12. The recombinant host cell of claim 11, which is a bacteria.

13. The recombinant host cell of claim 12, which is an Actinomycete.

14. The recombinant host cell of claim 13, which is Streptomyces.

15. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 9236-10201 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

16. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 15.

17. A recombinant vector comprising a chimeric gene according to claim 16.

18. A recombinant host cell comprising a chimeric gene according to claim 16.

19. The recombinant host cell of claim 18, which is a bacteria.

20. The recombinant host cell of claim 19, which is an Actinomycete.

21. The recombinant host cell of claim 20, which is Streptomyces.

22. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an enoyl reductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 10529-11428 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

23. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 22.

24. A recombinant vector comprising a chimeric gene according to claim 23.

25. A recombinant host cell comprising a chimeric gene according to claim 23.

26. The recombinant host cell of claim 25, which is a bacteria.

27. The recombinant host cell of claim 26, which is an Actinomycete.

28. The recombinant host cell of claim 27, which is Streptomyces.

29. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 11549-11764 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20

minutes at 65.degree. C.

30. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 29.

31. A recombinant vector comprising a chimeric gene according to claim 30.

32. A recombinant host cell comprising a chimeric gene according to claim 30.

33. The recombinant host cell of claim 32, which is a bacteria.

34. The recombinant host cell of claim 33, which is an Actinomycete.

35. The recombinant host cell of claim 34, which is Streptomyces.

36. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:2, amino acids 11-437 of SEQ ID NO:2, amino acids 543-864 of SEQ ID NO:2, amino acids 974-1273 of SEQ ID NO:2, and amino acids 1314-1385 of SEQ ID NO:2.

37. An isolated nucleic acid fragment according to claim 36, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 7610-11875 of SEQ ID NO:1, nucleotides 7643-8920 of SEQ ID NO:1, nucleotides 9236-10201 of SEQ ID NO:1, nucleotides 10529-11428 of SEQ ID NO:1, and nucleotides 11549-11764 of SEQ ID NO:1.

38. An isolated nucleic acid fragment according to claim 36, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 11-437 of SEQ ID NO:2.

39. An isolated nucleic acid fragment according to claim 38, wherein said nucleotide sequence is nucleotides 7643-8920 of SEQ ID NO:1.

40. An isolated nucleic acid fragment according to claim 36, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 543-864 of SEQ ID NO:2.

41. An isolated nucleic acid fragment according to claim 40, wherein said nucleotide sequence is nucleotides 9236-10201 of SEQ ID NO:1.

42. An isolated nucleic acid fragment according to claim 36, wherein said polypeptide comprises an enoyl reductase domain comprising amino acids 974-1273 of SEQ ID NO:2.

43. An isolated nucleic acid fragment according to claim 42, wherein said nucleotide sequence is nucleotides 10529-11428 of SEQ ID NO:1.

44. An isolated nucleic acid fragment according to claim 36, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 1314-1385 of SEQ ID NO:2.

45. An isolated nucleic acid fragment according to claim 44, wherein said nucleotide sequence is nucleotides 11549-11764 of SEQ ID NO:1.

46. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 36.

47. A recombinant vector comprising a chimeric gene according to claim 46.

48. A recombinant host cell comprising a chimeric gene according to claim 46.

49. The recombinant host cell of claim 48, which is a bacteria.
50. The recombinant host cell of claim 49, which is an Actinomycete.
51. The recombinant host cell of claim 50, which is Streptomyces.
52. An isolated polypeptide required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to a sequence selected from the group consisting of: nucleotides 7610-11875 of SEQ ID NO:1, nucleotides 7643-8920 of SEQ ID NO:1, nucleotides 9236-10201 of SEQ ID NO:1, nucleotides 10529-11428 of SEQ ID NO:1, and nucleotides 11549-11764 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
53. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 52.
54. The recombinant host cell of claim 53, which is a bacteria.
55. The recombinant host cell of claim 53, which is an Actinomycete.
56. The recombinant host cell of claim 53, which is Streptomyces.
57. An isolated polypeptide according to claim 52, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 7643-8920 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
58. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 57.
59. The recombinant host cell of claim 58, which is a bacteria.
60. The recombinant host cell of claim 59, which is an Actinomycete.
61. The recombinant host cell of claim 60, which is Streptomyces.
62. An isolated polypeptide according to claim 52, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 9236-10201 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
63. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 62.
64. The recombinant host cell of claim 63, which is a bacteria.
65. The recombinant host cell of claim 64, which is an Actinomycete.
66. The recombinant host cell of claim 65, which is Streptomyces.
67. An isolated polypeptide according to claim 52, wherein said polypeptide comprises an enoyl reductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 10529-11428 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

C.

68. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 67.

69. The recombinant host cell of claim 68, which is a bacteria.

70. The recombinant host cell of claim 69, which is an Actinomycete.

71. The recombinant host cell of claim 70, which is Streptomyces.

72. An isolated polypeptide according to claim 52, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 11549-11764 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

73. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 72.

74. The recombinant host cell of claim 73, which is a bacteria.

75. The recombinant host cell of claim 74, which is an Actinomycete.

76. The recombinant host cell of claim 75, which is Streptomyces.

77. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:2, amino acids 11-437 of SEQ ID NO:2, amino acids 543-864 of SEQ ID NO:2, amino acids 974-1273 of SEQ ID NO:2, and amino acids 1314-1385 of SEQ ID NO:2.

78. An isolated polypeptide according to claim 77, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 11-437 of SEQ ID NO:2.

79. An isolated polypeptide according to claim 77, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 543-864 of SEQ ID NO:2.

80. An isolated polypeptide according to claim 77, wherein said polypeptide comprises an enoyl reductase domain comprising amino acids 974-1273 of SEQ ID NO:2.

81. An isolated polypeptide according to claim 77, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 1314-1385 of SEQ ID NO:2.

82. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 77.

83. The recombinant host cell of claim 82, which is a bacteria.

84. The recombinant host cell of claim 83, which is an Actinomycete.

85. The recombinant host cell of claim 84, which is Streptomyces.

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Date: 10/9/2003
Time: 08:49:46**Application Number Information**Application Number: **09/567969** [Order This File Assignments](#)Examiner Number: **73397 / NASHED, NASHAAT**Filing Date: **05/10/2000**Group Art Unit: **1652**Effective Date: **05/10/2000**Class/Subclass: **435/183.000**Application Received: **05/11/2000**Lost Case: **NO**Patent Number: **6355457**

Interference Number:

Issue Date: **03/12/2002**Unmatched Petition: **NO**Date of Abandonment: **00/00/0000**L&R Code: Secrecy Code: **1**Attorney Docket Number: **4-30582F**Third Level Review: **NO**Secrecy Order: **NO**Status: **150 /PATENTED CASE**Status Date: **02/21/2002**Confirmation Number: **8324**Oral Hearing: **NO**Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09567969</u>	<u>9200</u>	03/08/2002	No Charge to Location	No Charge to Name	<u>WILLIAMS,NATASHA</u>	

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File: USPT

Mar 12, 2002

US-PAT-NO: 6355457

DOCUMENT-IDENTIFIER: US 6355457 B1

TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: March 12, 2002

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US-CL-CURRENT: 435/183, 435/189, 435/193, 435/195, 435/196, 435/232, 435/252.3,
435/252.35, 435/320.1, 530/300, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes at least one polypeptide required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 54935-62254 of SEQ ID NO:1, nucleotides 55028-56284 of SEQ ID NO:1, nucleotides 56600-57565 of SEQ ID NO:1, nucleotides 57593-58087 of SEQ ID NO:1, nucleotides 60362-61099 of SEQ ID NO:1, nucleotides 61211-61426 of SEQ ID NO:1, and nucleotides 61427-62254 of SEQ ID NO:1, under conditions of hybridization at 6.15.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 55028-56284 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and

washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

9. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.

10. A recombinant vector comprising a chimeric gene according to claim 9.

11. A recombinant host cell comprising a chimeric gene according to claim 9.

12. The recombinant host cell of claim 11, which is a bacteria.

13. The recombinant host cell of claim 12, which is an Actinomycete.

14. The recombinant host cell of claim 13, which is Streptomyces.

15. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 56600-57565 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

16. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 15.

17. A recombinant vector comprising a chimeric gene according to claim 16.

18. A recombinant host cell comprising a chimeric gene according to claim 16.

19. The recombinant host cell of claim 18, which is a bacteria.

20. The recombinant host cell of claim 19, which is an Actinomycete.

21. The recombinant host cell of claim 20, which is Streptomyces.

22. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 57593-58087 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

23. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 22.

24. A recombinant vector comprising a chimeric gene according to claim 23.

25. A recombinant host cell comprising a chimeric gene according to claim 23.

26. The recombinant host cell of claim 25, which is a bacteria.

27. The recombinant host cell of claim 26, which is an Actinomycete.

28. The recombinant host cell of claim 27, which is Streptomyces.

29. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 60362-61099 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20

minutes at 65.degree. C.

30. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 29.

31. A recombinant vector comprising a chimeric gene according to claim 30.

32. A recombinant host cell comprising a chimeric gene according to claim 30.

33. The recombinant host cell of claim 32, which is a bacteria.

34. The recombinant host cell of claim 33, which is an Actinomycete.

35. The recombinant host cell of claim 34, which is Streptomyces.

36. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 61211-61426 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

37. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 36.

38. A recombinant vector comprising a chimeric gene according to claim 37.

39. A recombinant host cell comprising a chimeric gene according to claim 37.

40. The recombinant host cell of claim 39, which is a bacteria.

41. The recombinant host cell of claim 40, which is an Actinomycete.

42. The recombinant host cell of claim 41, which is Streptomyces.

43. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a thioesterase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 61427-62254 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

44. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 43.

45. A recombinant vector comprising a chimeric gene according to claim 44.

46. A recombinant host cell comprising a chimeric gene according to claim 44.

47. The recombinant host cell of claim 46, which is a bacteria.

48. The recombinant host cell of claim 47, which is an Actinomycete.

49. The recombinant host cell of claim 48, which is Streptomnyces.

50. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:7, amino acids 32-450 of SEQ ID NO:7, amino acids 556-877 of SEQ ID NO:7, amino acids 887-1051 of SEQ ID NO:7, amino acids 1810-2055 of SEC ID NO:7, amino acids 2093-2164 of SEQ ID NO:7, and amino acids 2165-2439 of SEQ ID NO:7.

51. An isolated nucleic acid fragment according to claim 50, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 54935-62254 of SEQ ID NO:1, nucleotides 55028-56284 of SEQ ID NO:1, nucleotides 56600-57565 of SEQ ID NO:1, nucleotides 57593-58087 of SEQ ID NO:1, nucleotides 60362-61099 of SEQ ID NO:1, nucleotides 61211-61426 of SEQ ID NO:1 and nucleotides 61427-62254 of SEQ ID NO:1.
52. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 32-450 of SEQ ID NO:7.
53. An isolated nucleic acid fragment according to claim 52, wherein said nucleotide sequence is nucleotides 55028-56284 of SEQ ID NO:1.
54. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 556-877 of SEQ ID NO:7.
55. An isolated nucleic acid fragment according to claim 54, wherein said nucleotide sequence is nucleotides 56600-57565 of SEQ ID NO:1.
56. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a dehydratase domain comprising amino acids 887-1051 of SEQ ID NO:7.
57. An isolated nucleic acid fragment according to claim 56, wherein said nucleotide sequence is nucleotides 57593-58087 of SEQ ID NO:1.
58. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising amino acids 1810-2055 of SEQ ID NO:7.
59. An isolated nucleic acid fragment according to claim 50, wherein said nucleotide sequence is nucleotides 60362-61099 of SEQ ID NO:1.
60. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 2093-2164 of SEQ ID NO:7.
61. An isolated nucleic acid fragment according to claim 60, wherein said nucleotide sequence is nucleotides 61211-61426 of SEQ ID NO:1.
62. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a thioesterase domain comprising amino acids 2165-2439 of SEQ ID NO:7.
63. An isolated nucleic acid fragment according to claim 62, wherein said nucleotide sequence is nucleotides 61427-62254 of SEQ ID NO:1.
64. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 50.
65. A recombinant vector comprising a chimeric gene according to claim 64.
66. A recombinant host cell comprising a chimeric gene according to claim 64.
67. The recombinant host cell of claim 66, which is a bacteria.
68. The recombinant host cell of claim 67, which is an Actinomycete.
69. The recombinant host cell of claim 68, which is Streptomyces.

70. An isolated polypeptide required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to a sequence selected from the group consisting of: nucleotides 54935-62254 of SEQ ID NO:1, nucleotides 55028-56284 of SEQ ID NO:1, nucleotides 56600-57565 of SEQ ID NO:1, nucleotides 57593-58087 of SEQ ID NO:1, nucleotides 60362-61099 of SEQ ID NO:1, nucleotides 61211-61426 of SEQ ID NO:1, and nucleotides 61427-62254 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

71. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 70.

72. The recombinant host cell of claim 71, which is a bacteria.

73. The recombinant host cell of claim 72, which is an Actinomycete.

74. The recombinant host cell of claim 73, which is Streptomnyces.

75. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 55028-56284 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

76. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 75.

77. The recombinant host cell of claim 76, which is a bacteria.

78. The recombinant host cell of claim 77, which is an Actinomycete.

79. The recombinant host cell of claim 78, which is Streptomyces.

80. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 56600-57565 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

81. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 80.

82. The recombinant host cell of claim 81, which is a bacteria.

83. The recombinant host cell of claim 82, which is an Actinomycete.

84. The recombinant host cell of claim 83, which is Streptomyces.

85. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 57593-58087 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

86. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 85.

87. The recombinant host cell of claim 86, which is a bacteria.
88. The recombinant host cell of claim 87, which is an Actinomycete.
89. The recombinant host cell of claim 88, which is Streptomyces.
90. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 60362-61099 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
91. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 90.
92. The recombinant host cell of claim 91, which is a bacteria.
93. The recombinant host cell of claim 92, which is an Actinomycete.
94. The recombinant host cell of claim 93, which is Streptomyces.
95. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 61211-61426 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
96. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 95.
97. The recombinant host cell of claim 96, which is a bacteria.
98. The recombinant host cell of claim 97, which is an Actinomycete.
99. The recombinant host cell of claim 98, which is Streptomyces.
100. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a thioesterase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 61427-62254 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
101. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 99.
102. The recombinant host cell of claim 101, which is a bacteria.
103. The recombinant host cell of claim 102, which is an Actinomycete.
104. The recombinant host cell of claim 103, which is Streptomyces.
105. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:7, amino acids 32-450 of SEQ ID NO:7, amino acids 556-877 of SEQ ID NO:7, amino acids 887-1051 of SEQ ID NO:7, amino acids 1810-2055 of SEQ ID NO:7, amino acids 2093-2164 of SEQ ID NO:7, and amino acids 2165-2439 of SEQ ID NO:7.
106. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 32-450 of

SEQ ID NO:7.

107. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 556-877 of SEQ ID NO:7.

108. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a dehydratase domain comprising amino acids 887-1051 of SEQ ID NO:7.

109. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising amino acids 1810-2055 of SEQ ID NO:7.

110. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 2093-2164 of SEQ ID NO:7.

111. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a thioesterase domain comprising amino acids 2165-2439 of SEQ ID NO:7.

112. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 105.

113. The recombinant host cell of claim 112, which is a bacteria.

114. The recombinant host cell of claim 113, which is an Actinomycete.

115. The recombinant host cell of claim 114, which is Streptomyces.

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Application Number Information

Application Number: **09/568480** [Order This File Assignments](#)

Examiner Number: **73397 / NASHED, NASHAAT**

Filing Date: **05/10/2000**

Group Art Unit: **1652**

Effective Date: **05/10/2000**

Class/Subclass: **435/183.000**

Application Received: **05/11/2000**

Lost Case: **NO**

Patent Number: **6355458**

Interference Number:

Issue Date: **03/12/2002**

Unmatched Petition: **NO**

Date of Abandonment: **00/00/0000**

L&R Code: Secrecy Code: **1**

Attorney Docket Number: **4-30582D**

Third Level Review: **NO**

Secrecy Order: **NO**

Status: **150 /PATENTED CASE**

Status Date: **02/21/2002**

Confirmation Number: **7622**

Oral Hearing: **NO**

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09568480</u>	<u>9200</u>	04/03/2002	No Charge to Location	No Charge to Name	<u>BAIG,ARIF</u>	

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DOCUMENT-IDENTIFIER: US 6355458 B1

TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: March 12, 2002

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US-CL-CURRENT: 435/183, 435/189, 435/193, 435/232, 435/252.3, 435/252.35, 435/320.1, 530/300, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes at least one polypeptide required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 16251-21749 of SEQ ID NO:1, nucleotides 16269-17546 of SEQ ID NO:1, nucleotides 17865-18827 of SEQ ID NO:1, nucleotides 18855-19361 of SEQ ID NO:1, nucleotides 20565-21302 of SEQ ID NO:1, and nucleotides 21414-21626 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 16269-17546 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20

minutes at 65.degree. C.

9. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.

10. A recombinant vector comprising a chimeric gene according to claim 9.

11. A recombinant host cell comprising a chimeric gene according to claim 9.

12. The recombinant host cell of claim 11, which is a bacteria.

13. The recombinant host cell of claim 12, which is an Actinomycete.

14. The recombinant host cell of claim 13, which is Streptomyces.

15. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 17865-18827 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

16. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 15.

17. A recombinant vector comprising a chimeric gene according to claim 16.

18. A recombinant host cell comprising a chimeric gene according to claim 16.

19. The recombinant host cell of claim 18, which is a bacteria.

20. The recombinant host cell of claim 19, which is an Actinomycete.

21. The recombinant host cell of claim 20, which is Streptomyces.

22. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 18855-19361 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

23. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 22.

24. A recombinant vector comprising a chimeric gene according to claim 23.

25. A recombinant host cell comprising a chimeric gene according to claim 23.

26. The recombinant host cell of claim 25, which is a bacteria.

27. The recombinant host cell of claim 26, which is an Actinomycete.

28. The recombinant host cell of claim 28, which is Streptomyces.

29. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 20565-21302 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

30. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 29.
31. A recombinant vector comprising a chimeric gene according to claim 30.
32. A recombinant host cell comprising a chimeric gene according to claim 30.
33. The recombinant host cell of claim 32, which is a bacteria.
34. The recombinant host cell of claim 33, which is an Actinomycete.
35. The recombinant host cell of claim 34, which is Streptomyces.
36. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 21414-21626 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
37. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 36.
38. A recombinant vector comprising a chimeric gene according to claim 37.
39. A recombinant host cell comprising a chimeric gene according to claim 37.
40. The recombinant host cell of claim 39, which is a bacteria.
41. The recombinant host cell of claim 40, which is an Actinomycete.
42. The recombinant host cell of claim 41, which is Streptomyces.
43. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:4, amino acids 7-432 of SEQ ID NO:4, amino acids 539-859 of SEQ ID NO:4, amino acids 869-1037 of SEQ ID NO:4, amino acids 1439-1684 of SEQ ID NO:4, and amino acids 1722-1792 of SEQ ID NO:4.
44. An isolated nucleic acid fragment according to claim 43, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 16251-21749 of SEQ ID NO:1, nucleotides 16269-17546 of SEQ ID NO:1, nucleotides 17865-18827 of SEQ ID NO:1, nucleotides 18855-19361 of SEQ ID NO:1, nucleotides 20565-21302 of SEQ ID NO:1, and nucleotides 21414-21626 of SEQ ID NO:1.
45. An isolated nucleic acid fragment according to claim 43, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 7-432 of SEQ ID NO:4.
46. An isolated nucleic acid fragment according to claim 45, wherein said nucleotide sequence is nucleotides 16269-17546 of SEQ ID NO:1.
47. An isolated nucleic acid fragment according to claim 43, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 539-859 of SEQ ID NO:4.
48. An isolated nucleic acid fragment according to claim 47, wherein said nucleotide sequence is nucleotides 17865-18827 of SEQ ID NO:1.
49. An isolated nucleic acid fragment according to claim 43, wherein said

polypeptide comprises a dehydratase domain comprising amino acids 869-1037 of SEQ ID NO:4.

50. An isolated nucleic acid fragment according to claim 49, wherein said nucleotide sequence is nucleotides 18855-19361 of SEQ ID NO:1.

51. An isolated nucleic acid fragment according to claim 43, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising amino acids 1439-1684 of SEQ ID NO:4.

52. An isolated nucleic acid fragment according to claim 51, wherein said nucleotide sequence is nucleotides 20565-21302 of SEQ ID NO:1.

53. An isolated nucleic acid fragment according to claim 43, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 1722-1792 of SEQ ID NO:4.

54. An isolated nucleic acid fragment according to claim 53, wherein said nucleotide sequence is nucleotides 21414-21626 of SEQ ID NO:1.

55. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 43.

56. A recombinant vector comprising a chimeric gene according to claim 55.

57. A recombinant host cell comprising a chimeric gene according to claim 55.

58. The recombinant host cell of claim 57, which is a bacteria.

59. The recombinant host cell of claim 58, which is an Actinomycete.

60. The recombinant host cell of claim 59, which is Streptomyces.

61. An isolated polypeptide required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to a sequence selected from the group consisting of: nucleotides 16251-21749 of SEQ ID NO:1, nucleotides 16269-17546 of SEQ ID NO:1, nucleotides 17865-18827 of SEQ ID NO:1, nucleotides 18855-19361 of SEQ ID NO:1, nucleotides 20565-21302 of SEQ ID NO:1, and nucleotides 21414-21626 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

62. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 61.

63. The recombinant host cell of claim 62, which is a bacteria.

64. The recombinant host cell of claim 63, which is an Actinomycete.

65. The recombinant host cell of claim 64, which is Streptomyces.

66. An isolated polypeptide according to claim 61, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 16269-17546 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

67. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 66.

68. The recombinant host cell of claim 67, which is a bacteria.
69. The recombinant host cell of claim 68, which is an Actinomycete.
70. The recombinant host cell of claim 69, which is Streptomyces.
71. An isolated polypeptide according to claim 61, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 17865-18827 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
72. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 71.
73. The recombinant host cell of claim 72, which is a bacteria.
74. The recombinant host cell of claim 73, which is an Actinomycete.
75. The recombinant host cell of claim 74, which is Streptomyces.
76. An isolated polypeptide according to claim 61, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 18855-19361 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
77. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 76.
78. The recombinant host cell of claim 77, which is a bacteria.
79. The recombinant host cell of claim 78, which is an Actinomycete.
80. The recombinant host cell of claim 79, which is Streptomyces.
81. An isolated polypeptide according to claim 61, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 20565-21302 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
82. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 81.
83. The recombinant host cell of claim 82, which is a bacteria.
84. The recombinant host cell of claim 83, which is an Actinomycete.
85. The recombinant host cell of claim 84, which is Streptomyces.
86. An isolated polypeptide according to claim 61, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 21414-21626 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
87. A recombinant host cell comprising a recombinantly expressed polypeptide

according to claim 86.

88. The recombinant host cell of claim 87, which is a bacteria.

89. The recombinant host cell of claim 88, which is an Actinomycete.

90. The recombinant host cell of claim 89, which is Streptomyces.

91. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:4, amino acids 7-432 of SEQ ID NO:4, amino acids 539-859 of SEQ ID NO:4, amino acids 869-1037 of SEQ ID NO:4, amino acids 1439-1684 of SEQ ID NO:4, and amino acids 1722-1792 of SEQ ID NO:4.

92. An isolated polypeptide according to claim 91, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising amino acids 7-432 of SEQ ID NO:4.

93. An isolated polypeptide according to claim 91, wherein said polypeptide comprises an acyltransferase domain comprising amino acids 539-859 of SEQ ID NO:4.

94. An isolated polypeptide according to claim 91, wherein said polypeptide comprises a dehydratase domain comprising amino acids 869-1037 of SEQ ID NO:4.

95. An isolated polypeptide according to claim 91, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising amino acids 1439-1684 of SEQ ID NO:4.

96. An isolated polypeptide according to claim 91, wherein said polypeptide comprises an acyl carrier protein domain comprising amino acids 1722-1792 of SEQ ID NO:4.

97. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 91.

98. The recombinant host cell of claim 97, which is a bacteria.

99. The recombinant host cell of claim 98, which is an Actinomycete.

100. The recombinant host cell of claim 99, which is Streptomyces.

Day : Thursday
Date: 10/9/2003
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Application Number Information

Application Number: **09/568486** [Order This File Assignments](#)

Examiner Number: **73397 / NASHED, NASHAAT**

Filing Date: **05/10/2000**

Group Art Unit: **1652**

Effective Date: **05/10/2000**

Class/Subclass: **435/183.000**

Application Received: **05/11/2000**

Lost Case: **NO**

Patent Number: **6355459**

Interference Number:

Issue Date: **03/12/2002**

Unmatched Petition: **NO**

Date of Abandonment: **00/00/0000**

L&R Code: Secrecy Code: **1**

Attorney Docket Number: **4-30582E**

Third Level Review: **NO**

Secrecy Order: **NO**

Status: **150 /PATENTED CASE**

Status Date: **02/21/2002**

Confirmation Number: **8002**

Oral Hearing: **NO**

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09568486</u>	<u>9200</u>	03/08/2002	No Charge to Location	No Charge to Name	<u>WILLIAMS,NATASHA</u>	

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File: USPT

Mar 12, 2002

US-PAT-NO: 6355459

DOCUMENT-IDENTIFIER: US 6355459 B1

TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: March 12, 2002

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Cyr; Devon Dawn	Fuquay-Varina	NC		
Gorlach; Jorn	Durham	NC		

US-CL-CURRENT: 435/183; 435/189, 435/193, 435/232, 435/252.3, 435/252.35, 435/320.1, 536/23.2

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes at least one polypeptide required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 43524-54920 of SEQ ID NO:1, nucleotides 43626-44885 of SEQ ID NO:1, nucleotides 45204-46166 of SEQ ID NO:1, nucleotides 46950-47702 of SEQ ID NO:1, nucleotides 47811-48032 of SEQ ID NO:1, nucleotides 48087-49361 of SEQ ID NO:1, nucleotides 49680-50642 of SEQ ID NO:1, nucleotides 50670-51176 of SEQ ID NO:1, nucleotides 51534-52657 of SEQ ID NO:1, nucleotides 53697-54431 of SEQ ID NO:1, and nucleotides 54540-54758 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the

complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 43626-44885 of SEQ ID NO:1 and nucleotides 48087-49361 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

9. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.

10. A recombinant vector comprising a chimeric gene according to claim 9.

11. A recombinant host cell comprising a chimeric gene according to claim 9.

12. The recombinant host cell of claim 11, which is a bacteria.

13. The recombinant host cell of claim 12, which is an Actinomycete.

14. The recombinant host cell of claim 13, which is Streptomyces.

15. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 45204-46166 of SEQ ID NO:1 and nucleotides 49680-50642 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

16. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 15.

17. A recombinant vector comprising a chimeric gene according to claim 16.

18. A recombinant host cell comprising a chimeric gene according to claim 16.

19. The recombinant host cell of claim 18, which is a bacteria.

20. The recombinant host cell of claim 19, which is an Actinomycete.

21. The recombinant host cell of claim 20, which is Streptomyces.

22. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 50670-51176 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

23. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 22.

24. A recombinant vector comprising a chimeric gene according to claim 23.

25. A recombinant host cell comprising a chimeric gene according to claim 23.

26. The recombinant host cell of claim 25, which is a bacteria.

27. The recombinant host cell of claim 26, which is an Actinomycete.

28. The recombinant host cell of claim 27, which is Streptomyces.

29. An isolated nucleic acid fragment according to claim 1, wherein said

polypeptide comprises a methyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 51534-52657 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

30. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 29.

31. A recombinant vector comprising a chimeric gene according to claim 30.

32. A recombinant host cell comprising a chimeric gene according to claim 30.

33. The recombinant host cell of claim 32, which is a bacteria.

34. The recombinant host cell of claim 33, which is an Actinomycete.

35. The recombinant host cell of claim 34, which is Streptomyces.

36. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 46950-47702 of SEQ ID NO:1 and nucleotides 53697-54431 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

37. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 36.

38. A recombinant vector comprising a chimeric gene according to claim 37.

39. A recombinant host cell comprising a chimeric gene according to claim 37.

40. The recombinant host cell of claim 39, which is a bacteria.

41. The recombinant host cell of claim 40, which is an Actinomycete.

42. The recombinant host cell of claim 41, which is Streptomyces.

43. An isolated nucleic acid fragment according to claim 1, wherein said polypeptide comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 47811-48032 of SEQ ID NO:1 and nucleotides 54540-54758 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

44. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 43.

45. A recombinant vector comprising a chimeric gene according to claim 44.

46. A recombinant host cell comprising a chimeric gene according to claim 44.

47. The recombinant host cell of claim 46, which is a bacteria.

48. The recombinant host cell of claim 47, which is an Actinomycete.

49. The recombinant host cell of claim 48, which is Streptomyces.

50. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:6, amino acids 35-454 of SEQ ID NO:6, amino acids 561-881 of SEQ ID NO:6, amino acids 1143-1393 of SEQ ID NO:6, amino acids 1430-1503 of SEQ ID NO:6, amino acids 1522-1946 of SEQ ID NO:6, amino acids 2053-2373 of SEQ ID NO:6, amino acids 2383-2551 of SEQ ID NO:6, amino acids 2671-3045 of SEQ ID NO:6, amino acids 3392-3636 of SEQ ID NO:6, and amino acids 3673-3745 of SEQ ID NO:6.

51. An isolated nucleic acid fragment according to claim 50, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 43524-54920 of SEQ ID NO:1, nucleotides 43626-44885 of SEQ ID NO:1, nucleotides 45204-46166 of SEQ ID NO:1, nucleotides 46950-47702 of SEQ ID NO:1, nucleotides 47811-48032 of SEQ ID NO:1, nucleotides 48087-49361 of SEQ ID NO:1, nucleotides 49680-50642 of SEQ ID NO:1, nucleotides 50670-51176 of SEQ ID NO:1, nucleotides 51534-52657 of SEQ ID NO:1, nucleotides 53697-54431 of SEQ ID NO:1, and nucleotides 54540-54758 of SEQ ID NO:1.

52. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising an amino acid sequence selected from the group consisting of: amino acids 35-454 of SEQ ID NO:6 and amino acids 1522-1946 of SEQ ID NO:6.

53. An isolated nucleic acid fragment according to claim 52, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 43626-44885 of SEQ ID NO:1 and nucleotides 48087-49361 of SEQ ID NO:1.

54. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyltransferase domain comprising an amino acid sequence selected from the group consisting of: amino acids 561-881 of SEQ ID NO:6 and amino acids 2053-2373 of SEQ ID NO:6.

55. An isolated nucleic acid fragment according to claim 54, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 45204-46166 of SEQ ID NO:1 and nucleotides 49680-50642 of SEQ ID NO:1.

56. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a dehydratase domain comprising amino acids 2383-2551 of SEQ ID NO:6.

57. An isolated nucleic acid fragment according to claim 56, wherein said nucleotide sequence is nucleotides 50670-51176 of SEQ ID NO:1.

58. An isolated nucleic acid fragment according to claim 56, wherein said polypeptide comprises a methyltransferase domain comprising amino acids 2671-3045 of SEQ ID NO:6.

59. An isolated nucleic acid fragment according to claim 58, wherein said nucleotide sequence is nucleotides 51534-52657 of SEQ ID NO:1.

60. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 1143-1393 of SEQ ID NO:6 and amino acids 3392-3636 of SEQ ID NO:6.

61. An isolated nucleic acid fragment according to claim 60, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 46950-47702 of SEQ ID NO:1 and nucleotides 53697-54431 of SEQ ID NO:1.

62. An isolated nucleic acid fragment according to claim 50, wherein said polypeptide comprises an acyl carrier protein domain comprising an amino acid sequence selected from the group consisting of: amino acids 1430-1503 of SEQ ID NO:6 and amino acids 3673-3745 of SEQ ID NO:6.

63. An isolated nucleic acid fragment according to claim 62, wherein said nucleotide sequence is selected from the group consisting of: nucleotides 47811-48032 of SEQ ID NO:1 and nucleotides 54540-54758 of SEQ ID NO:1.
64. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 50.
65. A recombinant vector comprising a chimeric gene according to claim 64.
66. A recombinant host cell comprising a chimeric gene according to claim 64.
67. The recombinant host cell of claim 66, which is a bacteria.
68. The recombinant host cell of claim 67, which is an Actinomycete.
69. The recombinant host cell of claim 68, which is Streptomyces.
70. An isolated polypeptide required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to a sequence selected from the group consisting of: nucleotides 43524-54920 of SEQ ID NO:1, nucleotides 43626-44885 of SEQ ID NO:1, nucleotides 45204-46166 of SEQ ID NO:1, nucleotides 46950-47702 of SEQ ID NO:1, nucleotides 47811-48032 of SEQ ID NO:1, nucleotides 48087-49361 of SEQ ID NO:1, nucleotides 49680-50642 of SEQ ID NO:1, nucleotides 50670-51176 of SEQ ID NO:1, nucleotides 51534-52657 of SEQ ID NO:1, nucleotides 53697-54431 of SEQ ID NO:1, and nucleotides 54540-54758 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
71. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 70.
72. The recombinant host cell of claim 71, which is a bacteria.
73. The recombinant host cell of claim 72, which is an Actinomycete.
74. The recombinant host cell of claim 73, which is Streptomyces.
75. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 43626-44885 of SEQ ID NO:1 and nucleotides 48087-49361 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
76. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 75.
77. The recombinant host cell of claim 76, which is a bacteria.
78. The recombinant host cell of claim 77, which is an Actinomycete.
79. The recombinant host cell of claim 78, which is Streptomyces.
80. An isolated polypeptide according to claim 70, wherein said polypeptide comprises an acyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 45204-46166 of SEQ ID NO:1 and nucleotides 49680-50642 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and

washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

81. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 80.

82. The recombinant host cell of claim 81, which is a bacteria.

83. The recombinant host cell of claim 82, which is an Actinomycete.

84. The recombinant host cell of claim 83, which is Streptomyces.

85. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a dehydratase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 50670-51176 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

86. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 85.

87. The recombinant host cell of claim 86, which is a bacteria.

88. The recombinant host cell of claim 87, which is an Actinomycete.

89. The recombinant host cell of claim 88, which is Streptomyces.

90. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a methyltransferase domain and wherein the complement of said nucleotide sequence hybridizes to nucleotides 51534-52657 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

91. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 90.

92. The recombinant host cell of claim 91, which is a bacteria.

93. The recombinant host cell of claim 92, which is an Actinomycete.

94. The recombinant host cell of claim 93, which is Streptomyces.

95. An isolated polypeptide according to claim 70, wherein said polypeptide comprises a .beta.-ketoreductase domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 46950-47702 of SEQ ID NO:1 and nucleotides 53697-54431 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

96. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 95.

97. The recombinant host cell of claim 96, which is a bacteria.

98. The recombinant host cell of claim 97, which is an Actinomycete.

99. The recombinant host cell of claim 98, which is Streptomyces.

100. An isolated polypeptide according to claim 70, wherein said polypeptide

comprises an acyl carrier protein domain and wherein the complement of said nucleotide sequence hybridizes to a sequence selected from the group consisting of: nucleotides 47811-48032 of SEQ ID NO:1 and nucleotides 54540-54758 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

101. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 100.

102. The recombinant host cell of claim 101, which is a bacteria.

103. The recombinant host cell of claim 102, which is an Actinomycete.

104. The recombinant host cell of claim 103, which is Streptomyces.

105. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:6, amino acids 35-454 of SEQ ID NO:6, amino acids 561-881 of SEQ ID NO:6, amino acids 1143-1393 of SEQ ID NO:6, amino acids 1430-1503 of SEQ ID NO:6, amino acids 1522-1946 of SEQ ID NO: 6, amino acids 2053-2373 of SEQ ID NO:6, amino acids 2383-2551 of SEQ ID NO:6, amino acids 2671-3045 of SEQ ID NO:6, amino acids 3392-3636 of SEQ ID NO:6, and amino acids 3673-3745 of SEQ ID NO:6.

106. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoacyl-synthase domain comprising an amino acid sequence selected from the group consisting of: amino acids 35-454 of SEQ ID NO:6 and amino acids 1522-1946 of SEQ ID NO: 6.

107. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyltransferase domain comprising an amino acid sequence selected from the group consisting of: amino acids 561-881 of SEQ ID NO:6 and amino acids 2053-2373 of SEQ ID NO:6.

108. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a dehydratase domain comprising amino acids 2383-2551 of SEQ ID NO:6.

109. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a methyltransferase domain comprising amino acids 2671-3045 of SEQ ID NO:6.

110. An isolated polypeptide according to claim 105, wherein said polypeptide comprises a .beta.-ketoreductase domain comprising an amino acid sequence selected from the group consisting of: amino acids 1143-1393 of SEQ ID NO:6 and amino acids 3392-3636 of SEQ ID NO:6.

111. An isolated polypeptide according to claim 105, wherein said polypeptide comprises an acyl carrier protein domain comprising an amino acid sequence selected from the group consisting of: amino acids 1430-1503 of SEQ ID NO:6 and amino acids 3673-3745 of SEQ ID NO:6.

112. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 105.

113. The recombinant host cell of claim 112, which is a bacteria.

114. The recombinant host cell of claim 113, which is an Actinomycete.

115. The recombinant host cell of claim 114, which is Streptomyces.

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Application Number Information



Application Number: **09/568472** Order This File Assignments

Examiner Number: **73397 / NASHED, NASHAAT**

Filing Date: **05/10/2000**

Group Art Unit: **1652**

Effective Date: **05/10/2000**

Class/Subclass: **435/189.000**

Application Received: **05/11/2000**

Lost Case: **NO**

Patent Number: **6358719**

Interference Number:

Issue Date: **03/19/2002**

Unmatched Petition: **NO**

Date of Abandonment: **00/00/0000**

L&R Code: Secrecy Code: **1**

Attorney Docket Number: **4-30582G**

Third Level Review: **NO**

Secrecy Order: **NO**

Status: **150 /PATENTED CASE**

Status Date: **03/01/2002**

Confirmation Number: **8136**

Oral Hearing: **NO**

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09568472</u>	<u>9200</u>	04/03/2002	No Charge to Location	No Charge to Name	<u>BAIG,ARIF</u>	

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L3: Entry 15 of 38

File: USPT

Mar 19, 2002

US-PAT-NO: 6358719

DOCUMENT-IDENTIFIER: US 6358719 B1

TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: March 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Zirkle; Ross	Raleigh	NC		
Cyr; Devon Dawn	Fuquay-Varina	NC		
Gorlach; Jorn	Durham	NC		

US-CL-CURRENT: 435/189; 435/252.3, 435/252.35, 435/320.1, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a macrolactone oxidase required for the biosynthesis of an epothilone, wherein the complement of said nucleotide sequence hybridizes to nucleotides 62369-63628 of SEQ ID NO:1 under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a macrolactone oxidase of SEQ ID NO:8 that is required for the synthesis of an epothilone.
9. A nucleic acid fragment according to claim 8, wherein said nucleotide sequence is nucleotides 62369-63628 of SEQ ID NO: 1.
10. A chimeric gene comprising a heterologous promoter sequence operatively

linked to a nucleic acid fragment according to claim 8.

11. A recombinant vector comprising a chimeric gene according to claim 10.

12. A recombinant host cell comprising a chimeric gene according to claim 10.

13. The recombinant host cell of claim 12, which is a bacteria.

14. The recombinant host cell of claim 13, which is an Actinomycete.

15. The recombinant host cell of claim 14, which is Streptomyces.

16. An isolated polypeptide that is a macrolactone oxidase required for the biosynthesis of epothilone, wherein said polypeptide comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to nucleotides 62369-63628 of SEQ ID NO:1 under conditions of hybridization 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.

17. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 16.

18. The recombinant host cell of claim 17, which is a bacteria.

19. The recombinant host cell of claim 18, which is an Actinomycete.

20. The recombinant host cell of claim 19, which is Streptomyces.

21. An isolated polypeptide comprising SEQ ID NO:8.

22. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 21.

23. The recombinant host cell of claim 22, which is a bacteria.

24. The recombinant host cell of claim 23, which is an Actinomycete.

25. The recombinant host cell of claim 24, which is Streptomyces.

Day : Thursday
Date: 10/9/2003
Time: 08:49:27

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Application Number Information

Application Number: 09/567899

Assignments

Filing Date: 05/10/2000

Effective Date: 05/10/2000

Application Received: 05/11/2000

Patent Number: **6383787**

Issue Date: 05/07/2002

Date of Abandonment: 00/00/0000

Attorney Docket Number: 4-30582C

Status: 150 /PATENTED CASE

Confirmation Number: 7470

Title of Invention: **GENES FOR THE BIOSYNTHESIS OF EPOTHILONES**Examiner Number: 73397 / **NASHED, NASHAAT**

Group Art Unit: 1652

Class/Subclass: 435/183.000

Lost Case: NO

Interference Number:

Unmatched Petition: NO

L&R Code: Secrecy Code: 1

Third Level Review: NO

Secrecy Order: NO

Status Date: 04/19/2002

Oral Hearing: NO

Bar Code	PALM Location	Location Date	Charge to Loc	Charge to Name	Employee Name	Location
<u>09567899</u>	<u>1611</u>	04/24/2003	16X1	NASHED, NASHAAT	<u>1600,INCOMING MAIL</u>	CM1/07/C 14

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L3: Entry 14 of 38

File: USPT

May 7, 2002

US-PAT-NO: 6383787

DOCUMENT-IDENTIFIER: US 6383787 B1

**** See image for Certificate of Correction ****TITLE: Genes for the biosynthesis of epothilones

DATE-ISSUED: May 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Ligon; James Madison	Apex	NC		
Molnar; Istvan	Durham	NC		
Zirkle; Ross	Raleigh	NC		
Cyr; Devon Dawn	Fuquay-Varina	NC		
Gorlach; Jorn	Durham	NC		

US-CL-CURRENT: 435/183; 435/193, 435/252.3, 435/252.35, 435/320.1, 536/23.1, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a non-ribosomal peptide synthetase required for the biosynthesis of epothilone, wherein the complement of said nucleotide sequence hybridizes to nucleotides 11872-16104 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
2. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 1.
3. A recombinant vector comprising a chimeric gene according to claim 2.
4. A recombinant host cell comprising a chimeric gene according to claim 2.
5. The recombinant host cell of claim 4, which is a bacteria.
6. The recombinant host cell of claim 5, which is an Actinomycete.
7. The recombinant host cell of claim 6, which is Streptomyces.
8. An isolated nucleic acid fragment comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:3.
9. An isolated nucleic acid fragment according to claim 8, wherein said nucleotide sequence is nucleotides 11872-16104 of SEQ ID NO:1.

10. An isolated non-ribosomal peptide synthetase required for the biosynthesis of epothilone, which comprises an amino acid sequence encoded by a nucleotide sequence whose complement hybridizes to nucleotides 11872-16104 of SEQ ID NO:1, under conditions of hybridization at 65.degree. C. for 36 hours and washing 3 times at high stringency with 0.1.times.SSC and 0.5% SDS for 20 minutes at 65.degree. C.
11. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 10.
12. The recombinant host cell of claim 11, which is a bacteria.
13. The recombinant host cell of claim 12, which is an Actinomycete.
14. The recombinant host cell of claim 13, which is Streptomyces.
15. An isolated a non-ribosomal peptide synthetase compising the amino acid sequence of SEQ ID NO:3.
16. A recombinant host cell comprising a recombinantly expressed polypeptide according to claim 15.
17. The recombinant host cell of claim 16, which is a bacteria.
18. The recombinant host cell of claim 17, which is an Actinomycete.
19. The recombinant host cell of claim 18, which is Streptomyces.
20. A chimeric gene comprising a heterologous promoter sequence operatively linked to a nucleic acid fragment according to claim 8.
21. A recombinant vector comprising a chimeric gene according to claim 10.
22. A recombinant host cell comprising a chimeric gene according to claim 20.
23. The recombinant host cell of claim 22, which is a bacteria.
24. The recombinant host cell of claim 23, which is an Actinomycete.
25. The recombinant host cell of claim 24, which is Streptomyces.